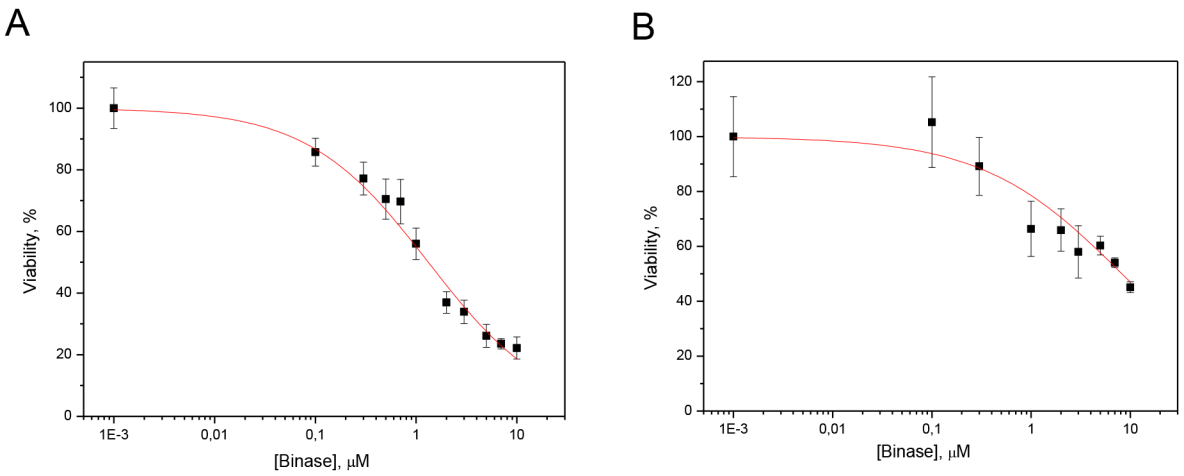
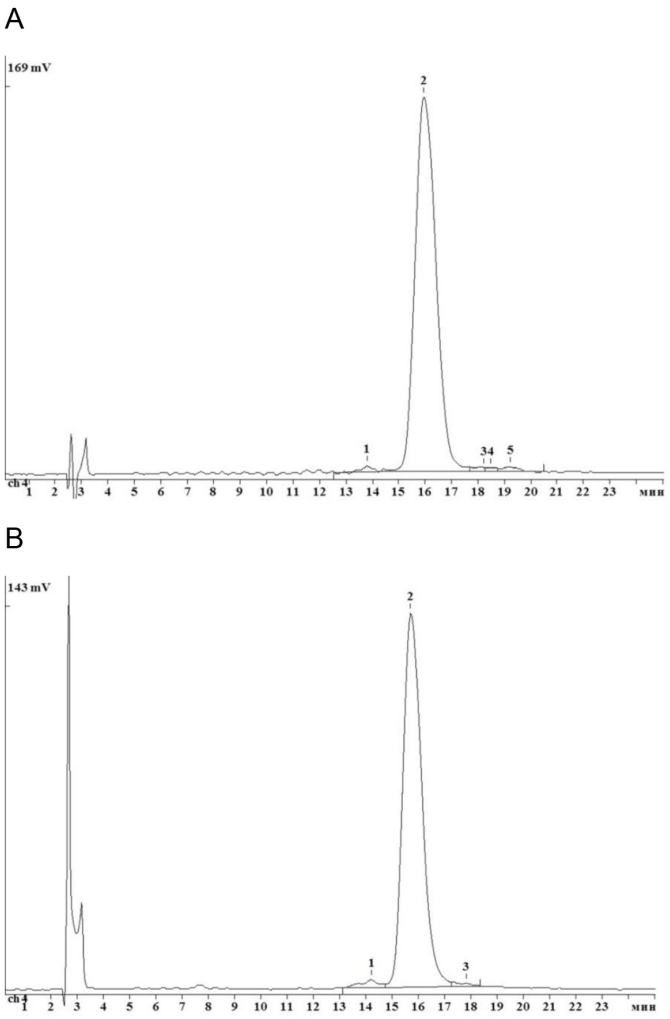
Binase treatment increases interferon sensitivity and apoptosis in SiHa cervical carcinoma cells by downregulating E6 and E7 human papilloma virus oncoproteins

SUPPLEMENTARY MATERIALS

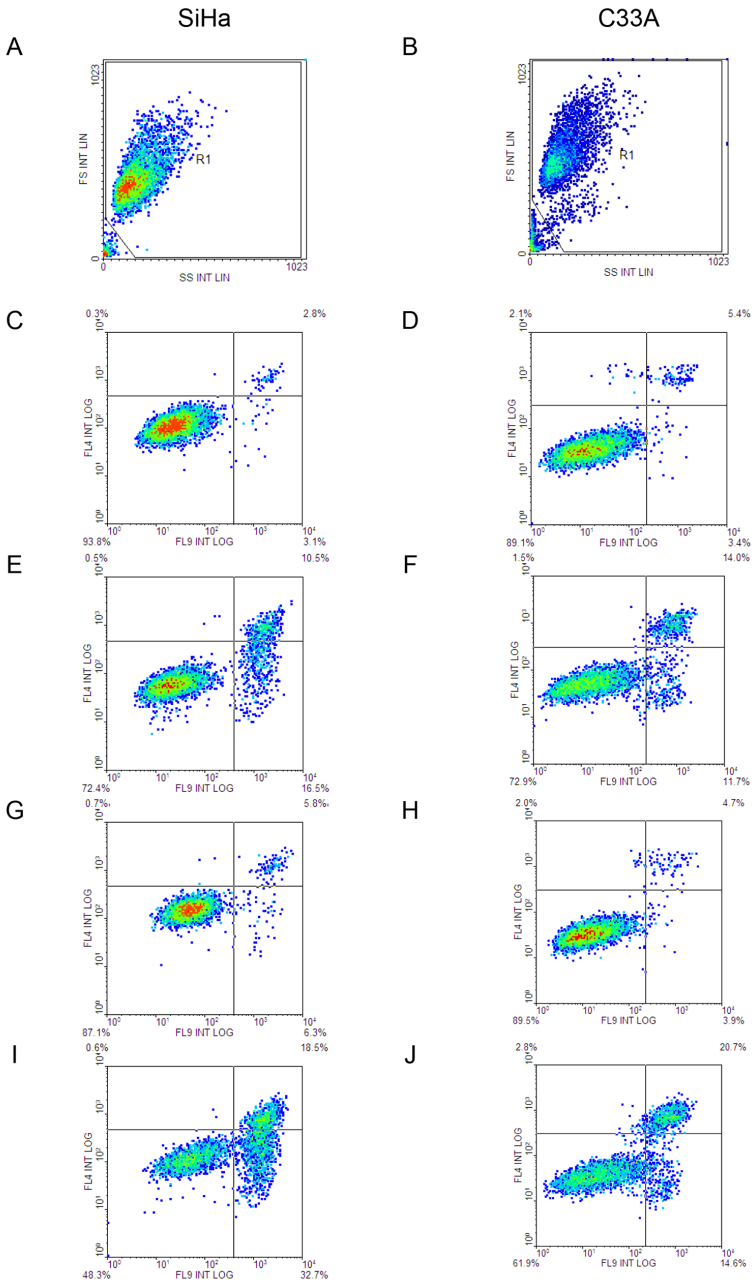


Supplementary Figure 1: Effect of binase on the viability of **(A)** SiHa and **(B)** C33A cells. Cell viability was measured by WST-1 test kit, 72 h after incubation with various concentrations of binase. Each value represents mean± SD of at least three independent experiments with triplicate samples. Values are expressed as a percentage relative to viability of the control without binase treatment.

Time (min)	Mobile phase A	Mobile phase B
0-1	72	28
1-5	67	33
5-20	63	37
20-30	57	43
30-33	40	60
33-37	40	60
37-39	72	28
39-60	72	28



Supplementary Figure 2: Chromatogram profile of IFNα2b preparation. Representative chromatogram profile of **(A)** IFNα2b preparation and **(B)** Reference IFNα2b (WHO standard, NIBSC95/566). Chromatographic separation conditions: column length 250 mm; column diameter 4 mm; column packed with Diaspher 300 C18 (6 μm); temperature: 45ºC; detection at 220 nm; flow rate 1 ml/min; mobile phase A: 30% acetonitrile, 0.2% TFA in water, mobile phase B: 80% acetonitrile, 0.2% TFA in water.



Supplementary Figure 3: Flow cytometry analysis of cytotoxic effects of binase (8 µM) and IFNα2b (100 ng/ml) treatments on SiHa and C33A cells at 48 h. (A,B) Flow cytometry density plot showing forward (FS) and side (SS) scatter of (A) SiHa and (B) C33A cells, describing their size and granularity. Cell population is marked by R1 gate. **(C-J)** Flow cytometry density plots showing Annexin-V Pacific Blue (FL9) versus propidium iodide (PI) (FL4) staining in (C,E,G,I) SiHa and (D,F,H,J) C33A cells. Lower left quadrant – AnnexinV$^-$PI$^-$ cells; lower right quadrant - AnnexinV$^+$ PI$^-$ cells; upper right quadrant – AnnexinV$^+$ PI$^+$ cells; upper left quadrant – AnnexinV$^-$ PI$^+$ cells. (C,D) Control cells; (E,F) cells treated by binase; (G,H) cells treated by IFNα2b and (I,J) cells treated by binase + IFNα2b.